

# Host Instar Susceptibility and Selection and Interspecific Competition of Three Introduced Parasitoids of the Mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae)

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**ABSTRACT** Three previously introduced parasitoids (*Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and *Pseudleptomastix mexicana* Noyes and Schauff [Hymenoptera: Encyrtidae]) of the mealybug *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) were studied for their host instar susceptibility and sex ratio, host instar selection, and interspecific competition in the laboratory. All three parasitoids were able to develop in the second instars, third-instar females, and adult females of *P. marginatus*. No progeny emerged from first-instar mealybugs. The proportion of female emergence was increased with increasing host size. Parasitoids selected their host instars for oviposition when they had a choice. Between second- and third-instar hosts, *A. papayae* and *P. mexicana* had significantly higher parasitism in second-instar mealybugs, whereas *A. loecki* had higher parasitism in the third-instar mealybugs. When competed with either one or two parasitoid species, *A. papayae* was significantly more successful in second-instar hosts and *A. loecki* was significantly more successful in third-instar mealybugs. *P. mexicana* was significantly less competitive when with *A. papayae* in both second and third instars, with *A. loecki* in third instars and with both *A. papayae* and *A. loecki* in second and third instars. Overall, *A. papayae* provided a better control of the host, when present singly or with the other two parasitoids. This information is important in evaluating the efficiency of *A. papayae*, *A. loecki*, and *P. mexicana* and understanding the outcome of their recovery and establishment in field studies conducted in Florida.

**KEY WORDS** parasitoids, hosts, biological control, parasitism

Classical biological control was identified as an important pest management practice for the mealybug *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), a polyphagous mealybug species that was first identified in the United States, in Florida in 1998 (Miller and Miller 2002). *P. marginatus* is a pest of many tropical and subtropical fruits, vegetables, and ornamental plants, including *Carica papaya* L. (papaya), *Hibiscus* spp. (hibiscus), *Citrus* spp. (citrus), *Persea americana* Mill. (avocado), and *Solanum melongena* L. (eggplant) (Miller and Miller 2002). This mealybug is believed to be native to Mexico and Central America (Noyes and Schauff 2003). This insect invaded the Caribbean islands in the early 1990s (Miller et al. 1999), Guam in 2002 (Meyerdirk et al. 2004), the Republic of Palau in 2003

(Muniappan et al. 2006), and several Hawaiian islands in 2004 (Heu et al. 2007).

Three solitary endoparasitoids *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and Menezes, and *Pseudleptomastix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) are currently mass reared in Puerto Rico to release, if needed, in mealybug-infested areas in the Caribbean and the Pacific islands of the United States by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) (Meyerdirk et al. 2004, Noyes and Schauff 2003). These three species of parasitoids were discovered from Mexico (Noyes and Schauff 2003). *A. papayae* and *A. loecki* were released with two other parasitoids (*Anagyrus californicus* Compere and *Pseudaphycus* sp.) in Bradenton (Manatee County), FL, in 2000 (Ngyuen 2000), but the outcome of these releases was not documented (Walker et al. 2006). *A. papayae*, *A. loecki*, and *P. mexicana* have been released in Miami-Dade and Broward Counties (Florida) for the first time in 2003 (Meyerdirk 2003; D. M. Amalin, unpublished observations). In July 2003, 1,400 *A. papayae*, 1,200 *A. loecki*, and 3,400 *P. mexicana* were released in Miami-Dade and Broward Counties, Florida, as a one-time release in 21 locations (D. M. Amalin, unpublished observa-

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tions). Field efficiency studies conducted in Florida showed a better adaptability of *A. papayae* over the heterospecific *A. loecki* and a lower efficiency and/or inability of field recovery of *P. mexicana* (Amarasekare et al. 2009). However, there is no information available on the host instar susceptibility, selection, and interspecific competition among these parasitoids.

Knowledge of host selection (King 1987) and interspecific competition (Schroder 1974) of parasitoids leads to better understanding of the population dynamics of the host and the parasitoids. Hence, this knowledge is important in evaluating and understanding the success of biological control and integrated pest management programs (King 1987). Understanding the host instar susceptibility and selection and interspecific competition of *A. papayae*, *A. loecki*, and *P. mexicana* is also important in explaining the results obtained in field efficiency studies carried out in Florida. The current study focused on the host instar susceptibility and parasitoid sex ratio, host instar selection, and interspecific competition of three previously introduced parasitoids of *P. marginatus*.

### Materials and Methods

**Mealybugs.** A colony of *P. marginatus* was maintained on red potatoes (sprouted) (*Solanum tuberosum* L.) (Ryan Potato Company, East Grand Forks, MN) in an environmental growth chamber (Percival I-36LL, Percival Scientific, Perry, NC) set at  $25^{\circ} \pm 1^{\circ}\text{C}$ ,  $65 \pm 2\%$  RH, and 12:12 (L:D)-h photoperiod. The colony was started with *P. marginatus* collected from infested papaya trees in Homestead, FL, in August 2006. (The initial mealybugs collected from the infested papaya field were observed for any parasitism, and the colony was started using the ovisacs produced by the field-collected adult females). Sprouted potatoes were infested weekly with *P. marginatus* ovisacs (3–5 ovisacs per potato) obtained from the colony mealybugs (Amarasekare et al. 2008).

To obtain mealybugs for experiments, ovisacs that were <24 h old were placed on a leaf in an arena. Leaves were obtained from containerized hibiscus plants maintained in a shadehouse. Arenas were prepared from a 9-cm-diameter petri dish with a 0.6-cm-diameter hole at the bottom. The stem (5 cm long) of a tender hibiscus (*Hibiscus rosa-sinensis* L.) leaf was inserted through the hole in the petri dish, and each dish was kept on a 162-ml translucent plastic soufflé cup (Georgia Pacific Dixie, Atlanta, GA) filled with water, which allowed the stem below the petiole to be in water.

These arenas were used for all studies, with ovisacs or mealybugs placed onto the hibiscus leaf in the arena. The gender of mealybugs was determined during the latter part of the second instar when males change their color from yellow to pink. Therefore, the gender was not determined for the first and second instars, but the third instars and adults used were females. (Preliminary studies showed that the third-instar [prepupa] and fourth-instar [pupa] males were

not selected for oviposition by the parasitoids; therefore, these stages were not used in this study.) Newly molted mealybugs, which were recognized by the size and presence of shed exuviae, were selected for all the experiments in this study to reduce the variation in host quality.

**Parasitoids.** Sprouted red potatoes with second- and third-instar mealybug colony nymphs were used for parasitoid rearing. Colonies of *A. papayae*, *A. loecki*, and *P. mexicana* were maintained in an insectary at  $25^{\circ} \pm 2^{\circ}\text{C}$  temperature, a 12:12 (L:D) photoperiod, and  $65 \pm 2\%$  RH. Colonies were initiated with parasitoids obtained from the Biological Control Laboratory of Puerto Rico Department of Agriculture through USDA-APHIS and were maintained in custom-made Plexiglas cages (30 × 30 × 30 cm; each with two cloth sleeves). Potatoes with parasitized mealybugs were moved to a new cage, and new mealybug-infested potatoes were supplied once per week. A solution of honey and water (1:1) was streaked on four pieces (5 × 5 cm) of Benchkote surface protector paper (Fisherbrand, Fisher, Pittsburgh, PA) attached to the cage using labeling tape (Fisherbrand, Fisher). Water was provided in two clear plastic 73.9-ml containers (Tristate Molded Plastic, North Dixon, KY) per cage. In each container, a 1-cm-diameter hole was made in the center of the lid, and a 7.6-cm-long piece of cotton roll (TIDI Products, Neenah, WI) was inserted through the hole to allow parasitoids to access water.

**Mated Female Parasitoids.** To obtain mated female parasitoids, newly emerged females of each species were placed singly in disposable glass culture tubes (1.2 × 7.5 cm; Fisherbrand, Fisher, Pittsburgh, PA) and closed with two-ply tissue (Kimwipes EX-L, Kimberly-Clerk Global Sales, Roswell, GA) secured with a piece of rubber tubing (0.95 × 2.5 cm; Fisherbrand, Fisher). Five newly emerged males were placed in each tube with a female and were allowed to mate for 24 h. A streak of honey and water (1:1) was provided for each tube. (Preliminary studies conducted to obtain mated female parasitoids have shown that using the above method could obtain mated parasitoids. A progeny produced by a mated parasitoid consisted of both males and females, whereas an unmated female can produce male-only progeny.) After 24 h, males were removed and females were used in experiments. Females were separated from males by presence of ovipositor, body size, and variation in antennae (Noyes 2000, Noyes and Schauff 2003). All experiments were carried out at  $25^{\circ} \pm 2^{\circ}\text{C}$  temperature, a 12:12 (L:D)-h photoperiod, and  $65 \pm 2\%$  RH.

**Host Instar Susceptibility and Sex Ratio.** Susceptibility of first and second instars, third instars (females), and adult females were determined in no-choice tests. Ten individuals (comprising one replicate) of each instar were placed on a hibiscus leaf in an arena (using a paintbrush [number 000, American Painter 4000, Loew-Cornell, Englewood Cliffs, NJ]), which was covered with a piece of black cotton cloth to encourage mealybugs to settle. After 24 h, a

streak of honey and water (1:1) was placed on the inside of the lid, and a single mated female parasitoid was placed on the leaf in each dish. The arena was covered with a piece ( $15 \times 15$  cm) of chiffon cloth material (Jo-Ann Fabrics and Crafts, Miami, FL), and secured with a rubber band to prevent parasitoid escape. Each parasitoid was allowed to oviposit for 24 h, and then it was removed. Mummified mealybugs were placed individually in disposable glass culture tubes and were secured as above. Gender and number of parasitoids that emerged were determined for the sex ratio and proportion of parasitism. There were 25 replicates for each mealybug instar and parasitoid combination.

**Host Instar Selection.** Two-at-a-time choice tests were conducted to determine parasitoid selection of host instar. A mated female parasitoid was given one of the following three treatments: five individuals each of second instars and third-instar females, second instars and adult females, and third instars and adult females, with a total of 10 individuals per dish. After a 24-h period of oviposition, the female parasitoid was removed and the two groups per treatment were separated by moving one group to a new hibiscus leaf prepared at the same time as the original leaves. Host instars were separated to facilitate identification of parasitoid selection between the two groups offered, and the mummified mealybugs were moved to individual glass culture tubes. The number and the gender of parasitoids emerging from the mummified mealybugs were recorded for each parasitoid species. Information from Noyes (2000) and Noyes and Schauff (2003) was used in identifying different parasitoid species. For verification of identification, parasitoid samples from each species were sent to Systematic Entomology Laboratory, USDA-Agricultural Research Service (ARS). Each mealybug combination for each parasitoid species had 25 replicates.

**Interspecific Competition.** Interspecific competition of parasitoids was studied using 10 second instars or 10 third-instar females. Each host instar was placed separately on a hibiscus leaf arena. The four parasitoid combinations used were *A. papayae* and *A. loecki*; *A. papayae* and *P. mexicana*; *A. loecki* and *P. mexicana*; and *A. papayae*, *A. loecki*, and *P. mexicana*. A mated female of each parasitoid species was used in all combinations. They were allowed to parasitize for 24 h, and were then removed. The mealybugs were allowed to mummify on the hibiscus leaves, and mummified mealybugs were treated as described above. The number and the species of parasitoids that emerged from each combination in each mealybug instar were counted. The mean percent parasitism was calculated from the 10 mealybugs used for each host instar in each parasitoid combination. Each parasitoid combination for each host instar had 25 replicates.

**Statistical Analysis.** The experimental design was completely random for all experiments. A two-way analysis of variance was performed using a general linear model (PROC GLM) of SAS (SAS Institute 1999) to find the interaction between parasitoids and host in host instar susceptibility and sex ratio experi-

**Table 1.** Mean percent parasitism ( $\pm$ SEM) by *A. papayae*, *A. loecki*, and *P. mexicana* reared in different developmental instars of *P. marginatus* to evaluate host instar susceptibility using no-choice tests

Parasitoid	Mean percent parasitism (%) for instars of <i>P. marginatus</i>		
	Second instar	Third-instar female	Adult female
<i>A. papayae</i>	82.8 $\pm$ 2.1aA	71.2 $\pm$ 2.6bB	60.8 $\pm$ 2.9bC
<i>A. loecki</i>	41.2 $\pm$ 2.8cB	82.4 $\pm$ 1.9aA	74.8 $\pm$ 3.2aA
<i>P. mexicana</i>	70.8 $\pm$ 1.9bA	50.8 $\pm$ 2.5cB	40.8 $\pm$ 3.6cB
ANOVA results			
Source	F	df	P
Model	36.32	8, 216	<0.0001
Parasitoid	34.06	2, 216	<0.0001
Instar	8.67	2, 216	0.0002
Parasitoid $\times$ instar	51.27	4, 216	<0.0001

Means within a column followed by the same lowercase letters, and means within a row followed by the same uppercase letters are not significantly different at  $\alpha = 0.05$  (least square means test).  $n = 25$ .

ments. Means were compared at  $P = 0.05$  significance level using least square means of SAS (SAS Institute 1999). For host instar selection tests, means of two stages of *P. marginatus* for each parasitoid species were compared using a  $t$  test (PROC TTEST) of SAS (SAS Institute 1999). In interspecific competition studies, PROC GLM was used for significance among the parasitoids and means were compared at  $P = 0.05$  significance level using least square means. Proportions of females (sex ratio) and percentages of parasitism were arcsine square-root transformed using

$$p' = \arcsin \sqrt{p}$$

(where  $P$  = proportion of female or percentage of parasitism) to adjust the variances (Zar 1984) before analysis of variance.

**Voucher Specimens.** Voucher specimens of *P. marginatus*, *A. papayae*, *A. loecki*, and *P. mexicana* were deposited in the Entomology and Nematology Department insect collection at Tropical Research and Education Center (University of Florida).

## Results

**Host Instar Susceptibility and Sex Ratio.** All three parasitoids were able to complete development in second-instar, third-instar female, and adult female mealybugs (Table 1). There was a significant interaction between parasitoids and host instars ( $F = 51.27$ ;  $df = 4, 216$ ;  $P < 0.0001$ ). Each parasitized mealybug yielded a single parasitoid. The mean percent parasitism decreased with increasing host size (second instar, third-instar female, and adult female) for both *A. papayae* and *P. mexicana* (82.8, 71.2, and 60.8%, and 70.8, 50.8, and 40.8%, respectively). No parasitoids emerged from first instars for any of the three species. More than 80% of the first-instar mealybugs tested from all three parasitoid species were able to complete their development to adults.

There was a significant interaction between parasitoids and host instars on proportion of progeny fe-

**Table 2.** Mean proportion of females (sex ratio) ( $\pm$ SEM) of *A. papayae*, *A. loecki*, and *P. mexicana* reared in different developmental instars of *P. marginatus* to evaluate host instar susceptibility using no-choice tests

Parasitoid	Mean proportion of females (sex ratio) for instars of <i>P. marginatus</i>		
	Second instar	Third-instar female	Adult female
<i>A. papayae</i>	0.50 $\pm$ 0.01aB	0.56 $\pm$ 0.01aA	0.57 $\pm$ 0.01aA
<i>A. loecki</i>	0.40 $\pm$ 0.01cC	0.51 $\pm$ 0.01bB	0.54 $\pm$ 0.01bA
<i>P. mexicana</i>	0.48 $\pm$ 0.01bC	0.55 $\pm$ 0.01aB	0.56 $\pm$ 0.01aA
ANOVA results			
Source	<i>F</i>	df	<i>P</i>
Model	72.34	8, 216	<0.0001
Parasitoid	73.68	2, 216	<0.0001
Instar	196.98	2, 216	<0.0001
Parasitoid $\times$ instar	9.35	4, 216	<0.0001

Means within a column followed by the same lowercase letters, and means within a row followed by the same uppercase letters are not significantly different at  $\alpha = 0.05$  (least square means test).  $n = 25$ .

males (sex ratio;  $F = 9.35$ ;  $df = 4, 216$ ;  $P < 0.0001$ ). The proportion of progeny females increased with increasing host size (Table 2). There was an unbiased sex ratio for *A. papayae* that emerged from second-instar hosts (0.50), but male-biased sex ratios for *A. loecki* (0.40) and *P. mexicana* (0.48). Among parasitoids that emerged from third instars and adult females, all had female-biased sex ratios.

**Host Instar Selection.** When parasitoids were given a choice for host instar selection for oviposition, *A. papayae* (77.6%) and *P. mexicana* (69.6%) selected second instars ( $t = 6.54$ ,  $df = 48$ ,  $P = <0.0001$ , and  $t = 6.73$ ,  $df = 48$ ,  $P = <0.0001$ , respectively) over third-instar or adult females, whereas *A. loecki* selected third-instar (76.0%) ( $t = -13.6$ ,  $df = 48$ ,  $P = <0.0001$ ) or adult females (68.8%) ( $t = 9.11$ ,  $df = 48$ ,  $P = <0.0001$ ) over second instars (Table 3). Between third-instar and adult females, *A. papayae* (60.0%) ( $t = -1.81$ ,  $df = 48$ ,  $P = 0.0471$ ) and *A. loecki* (79.2%) ( $t = -4.58$ ,  $df = 48$ ,  $P = <0.0001$ ) selected third instars, whereas *P. mexicana* had no selection ( $t = -1.85$ ,  $df = 48$ ,  $P = <0.0691$ ).

**Interspecific Competition.** Host instar and parasitoid combination affected the success of parasitism (Table 4). There was a significant interaction between

host instar and parasitoid combination ( $F = 44.09$ ;  $df = 8, 432$ ;  $P < 0.0001$ ). *A. papayae* (69.6, 78.4, and 59.6%) was always more successful than *P. mexicana* (20.0, 50.4, and 23.6%) in second instars, whether it was just the two parasitoids or with all three parasitoids. *A. loecki* was better than *A. papayae* or *P. mexicana* when present with just the two parasitoids or with all three parasitoids (54.8, 75.2, and 47.6%) in third-instar host stages. *P. mexicana* was significantly more successful than *A. loecki* in second-instar hosts when present with just the two parasitoids (50.4%) or with all three parasitoids (23.6%).

## Discussion

All host instars except the first were susceptible for parasitism by the three parasitoids. Parasitoids selected their host instars for oviposition when they had a choice. Interspecific competition was observed when forced to compete with either one or two parasitoid species.

First-instar nymphs of *P. marginatus* were not susceptible to *A. papayae*, *A. loecki*, or *P. mexicana*. Although the first instars were not dissected to find parasitoid eggs in this study, >80% of the first-instar mealybugs used were able to develop into adults. This makes the first instars, which are  $\approx 0.4$  mm in size (Miller and Miller 2002), unsuitable for successful parasitoid reproduction. Size of the host is one of the important factors that solitary endoparasitoids consider when they select host stages for oviposition (Vinson and Iwantsch 1980). Increased host size translates into both increased male and female fitness (Charnov et al. 1981). For females, this measure is the lifetime production of eggs, and for males, it is the length of life (Charnov et al. 1981).

A parasitoid's biology may be greatly influenced by the quality of the host (Doutt 1959). Host stage is an important ecological variable, which may have an influence on a parasitoid's rate of attack, survival of its immature stages, and sex ratio of its offspring (Waage 1986). In similar parasitoid behavioral studies, first-instar *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae) were preferred for host feeding by the parasitoid *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) (Bokonon-Ganta et al. 1995). Para-

**Table 3.** Mean percent parasitism ( $\pm$ SEM) by *A. papayae*, *A. loecki*, and *P. mexicana* reared in different instar combinations of *P. marginatus* to evaluate host instar selection using choice tests

Host instar combination of <i>P. marginatus</i>		Parasitoid	Mean percent parasitism		<i>T</i> statistics		
Stage 1	Stage 2		Stage 1	Stage 2	<i>t</i>	df	<i>P</i>
Second instar	Third-instar female	<i>A. papayae</i>	77.6 $\pm$ 1.8	58.4 $\pm$ 2.6	6.54	48	<0.0001
		<i>A. loecki</i>	30.4 $\pm$ 2.9	76.0 $\pm$ 2.0	-13.60	48	<0.0001
		<i>P. mexicana</i>	69.6 $\pm$ 2.6	40.8 $\pm$ 3.6	6.73	48	<0.0001
Second instar	Adult female	<i>A. papayae</i>	76.8 $\pm$ 1.9	50.4 $\pm$ 4.2	-5.98	48	<0.0001
		<i>A. loecki</i>	32.0 $\pm$ 3.1	68.8 $\pm$ 2.6	9.11	48	<0.0001
		<i>P. mexicana</i>	68.8 $\pm$ 2.6	32.0 $\pm$ 3.3	-8.78	48	<0.0001
Third instar	Adult female	<i>A. papayae</i>	60.0 $\pm$ 3.1	48.0 $\pm$ 5.0	-1.81	48	0.0471
		<i>A. loecki</i>	79.2 $\pm$ 0.8	64.8 $\pm$ 3.1	-4.58	48	<0.0001
		<i>P. mexicana</i>	41.6 $\pm$ 3.4	32.8 $\pm$ 3.2	-1.85	48	0.0691

$n = 25$ .



**Table 4.** Mean percent parasitism ( $\pm$ SEM) by combinations of *A. papayae*, *A. loecki*, and *P. mexicana* reared in second- and third-instar *P. marginatus* to evaluate interspecific competition of parasitoids

Instar of <i>P. marginatus</i>	Combination of parasitoids			Mean percent parasitism		
	Parasitoid 1	Parasitoid 2	Parasitoid 3	<i>A. papayae</i>	<i>A. loecki</i>	<i>P. mexicana</i>
Second instar	<i>A. papayae</i>	<i>A. loecki</i>	–	69.6 $\pm$ 2.3a	19.6 $\pm$ 1.7b	–
	<i>A. papayae</i>	–	<i>P. mexicana</i>	78.4 $\pm$ 1.2a	–	20.0 $\pm$ 1.3b
	–	<i>A. loecki</i>	<i>P. mexicana</i>	–	40.4 $\pm$ 3.5b	50.4 $\pm$ 3.3a
	<i>A. papayae</i>	<i>A. loecki</i>	<i>P. mexicana</i>	59.6 $\pm$ 2.9a	14.8 $\pm$ 1.8c	23.6 $\pm$ 2.1b
Third-instar female	<i>A. papayae</i>	<i>A. loecki</i>	–	42.4 $\pm$ 3.3b	54.8 $\pm$ 3.5a	–
	<i>A. papayae</i>	–	<i>P. mexicana</i>	59.2 $\pm$ 4.3a	–	35.6 $\pm$ 3.5b
	–	<i>A. loecki</i>	<i>P. mexicana</i>	–	75.2 $\pm$ 2.7a	20.4 $\pm$ 1.4b
	<i>A. papayae</i>	<i>A. loecki</i>	<i>P. mexicana</i>	38.8 $\pm$ 4.4b	47.6 $\pm$ 4.5a	11.2 $\pm$ 0.6c
ANOVA results						
Source	<i>F</i>	<i>df</i>	<i>P</i>			
Model	49.8	17, 432	<0.0001			
Instar	0.71	1, 432	0.4887			
Combination	61.67	8, 432	<0.0001			
Instar $\times$ combination	44.09	8, 432	<0.0001			

Means within a row followed by the same lowercase letters are not significantly different at  $\alpha = 0.05$  (least square means test).  $n = 25$ .

parasitoids such as *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) can oviposit in first instars of *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), and in most situations, the ovipositor of *A. kamali* remained stuck within the first-instar host, precluding further foraging by the parasitoid (Sagarra and Vincent 1999). This behavior was also shown in the second instars of *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), which were often impaled on the ovipositor of *Anagyrus pseudococchi* (Girault) (Hymenoptera: Encyrtidae) (Islam and Copland 1997). In this study, host feeding and ovipositing behaviors and larval development of *A. papayae*, *A. loecki*, and *P. mexicana* were not evaluated.

Although all three parasitoid species were able to complete their life cycle in the second-instar hosts, only *A. papayae* produced a higher proportion of female progeny. Second-instar mealybugs may have enough resources for the smallest of the three parasitoids, which is *A. papayae* (Noyes 2000, Noyes and Schauff 2003). In contrast, *A. loecki*, the largest of the three parasitoids (Noyes 2000, Noyes and Schauff 2003), had male-biased sex ratio in second-instar hosts and female-biased sex ratio in third-instar and adult female mealybugs. Host selection behavior is most important in determining the sex ratio of arrhenotokous parasitoids, which show a haplodiploid sex determination mechanism (King 1987). A female parasitoid can manipulate the offspring sex ratio at oviposition by regulating fertilization (King 1987). A particular host size may be more suitable for the development of one sex, so that, in general, a female-biased offspring sex ratio is produced from the larger hosts and a male-biased sex ratio from the smaller hosts (King 1987). A female-biased progeny sex ratio is a desirable characteristic for an efficient biological control agent. By choosing a larger host, the parasitoid accessed a larger food supply and increased the fitness of its progeny. In larger hosts, a female-biased progeny was recorded for other parasitoids (King 1987). The solitary endoparasitoid, *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae), which was able to oviposit in second-instar

nymphs of *Phenacoccus herreni* Cox and Williams (Hemiptera: Pseudococcidae), also recorded a considerably higher proportion of males in the second instar than in the larger instars of *P. herreni* (Bertschy et al. 2000).

Interspecific competition was observed when *A. papayae*, *A. loecki*, and *P. mexicana* competed for host mealybugs. The outcome of the competition was affected by the host instar they parasitized. In the second-instar hosts, *A. papayae* had  $\approx 60\%$  parasitism level and a female-biased sex ratio, indicating its superior ability to compete. This may be one reason that *A. papayae* was well established and recovered from the field in the Republic of Palau (Muniappan et al. 2006). In field studies conducted in Florida, both *A. papayae* and *A. loecki* were recovered, but *P. mexicana* was not (Amarasekare et al. 2009). *P. mexicana* was also not recovered in the field studies conducted in the Republic of Palau (Muniappan et al. 2006). The low parasitism levels by *P. mexicana* observed from this study suggest that *P. mexicana* may be less competitive than the other two parasitoids.

Sympatric parasitoid species that share the same host species may be competitors (van Strien-van Liempt 1983). Intensive studies of parasitic complexes in connection with biological control programs have shown that interspecific competition can be extremely important (Schroder 1974). The greater the part of the host population that is exploited by both species, the more they will affect each other's population density. Their competitive abilities then, among other factors, determine their relative abundance (van Strien-van Liempt 1983). In solitary insect parasitoids, generally only one offspring survives in a host (Vinson 1976). Females normally deposit one egg per host and reduce the host availability to both conspecific and heterospecific parasitoids. Successful oviposition by a female depends on how efficient she is in finding and parasitizing unparasitized hosts. This leads to interspecific competition among the parasitoids in classical biological control where more than one parasitoid species is used (Lawrence 1981).

In addition to being a parasitoid of *P. marginatus*, *A. loecki* can develop in *Dysmicoccus hurdi* and *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) (Noyes 2000). *P. madeirensis* is one of the commonly found mealybug species in southern Florida. There is no information on other host mealybugs for *A. papayae* or *P. mexicana* or the host preference by *A. loecki* between *P. madeirensis* and *P. marginatus*. Not being host specific and having less competitive ability may have caused the lower parasitism of *A. loecki* found in the field studies conducted in Florida (Amarasekare et al. 2009).

Overall, *A. papayae* possesses suitable characteristics with regard to host instar selection and interspecific competition with the other two parasitoids of *P. marginatus*. It showed better adaptability by being able to oviposit in second-instar to adult female mealybugs with female-biased sex ratio. In mass rearing of parasitoids, second-instar *P. marginatus* is a suitable host for *A. papayae* and *P. mexicana*, whereas third-instar females are suitable for rearing of *A. loecki*. The information obtained in this study will be helpful in explaining the better adaptability of *A. papayae* observed in the field over the heterospecific *A. loecki*. This information also provides possible explanations for the better adaptability of *A. papayae* over *A. loecki* and the lower efficiency and/or inability of the field recovery of *P. mexicana*.

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